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EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	**PAPER NUMBER**
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/839,583	COLOSI, PETER	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 September 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-55* is/are pending in the application.

4a) Of the above claim(s) *1-23 and 41-49* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *27-39 and 50-55* is/are rejected.

7)☒ Claim(s) *24-26 and 40* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *14 August 2001* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *1/18/02, 2/20/03*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-55 are pending in the instant application.

Election/Restrictions

Applicant's election without traverse of Group II (claims 24-40 and 50-55) in the reply filed on 9/26/2005 is acknowledged.

Claims 1-23 and 41-49 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention. An examination on the merits of claims 24-40 and 50-55 follows.

Oath/Declaration

The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:
It was not executed in accordance with either 37 CFR 1.66 or 1.68. The declaration, filed 4/20/2001, has not been signed by Peter Colosi.

Information Disclosure Statement

Receipt of information disclosure statements, filed on 1/18/2002 and 2/20/2003, is acknowledged. The signed and initialed PTO 1449s have been mailed with this action.

Drawings

The drawings are objected to as failing to comply with 37 CFR 1.84(p)(5) because they include the following reference character(s) not mentioned in the description: panels A, B and C of Figure 7 are not separately described in the brief description of the drawing. Corrected drawing sheets in compliance with 37 CFR 1.121(d), or amendment to the specification to add the reference character(s) in the description in compliance with 37 CFR 1.121(b) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Objections

Claims 24, 25 and 26 are objected to because of the following informalities: the claims depend from withdrawn claims. Appropriate correction is required.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 27-33 and 37-39 are rejected under 35 U.S.C. 102(e) as being anticipated by Dong et al (US Patent No. 6,686,200; see the entire reference).

Regarding claims 27 and 31, Dong et al teach a method of producing recombinant AAV (rAAV) virions, comprising the following steps: (i) introducing a recombinant AAV vector into a host cell, (ii) infecting the cell with recombinant adenovirus capable of expressing one or more AAV proteins, and (iii) culturing the cell under conditions and for a period of time effective to allow the cell to produce recombinant AAV virions (e.g. column 7, lines 45-61). Dong et al teach recombinant adenovirus vectors containing the AAV *rep* gene or the AAV *cap* gene (e.g. Figure 5; Example III). Thus, the step of infecting the cell with recombinant adenovirus encompasses the infection of the cell with the adenoviral vector comprising the AAV *rep* gene and infecting the cell with the adenoviral vector comprising the AAV *cap* gene and adenoviral accessory functions.

Regarding claim 28, Dong et al teach the introduction of AAV by infection (e.g. column 25, lines 30-61).

Regarding claims 29 and 30, Dong et al teach that the recombinant AAV vector sequences may be maintained in the host cells as integrated or episomal copies (e.g. column 5, lines 59-67; column 6, lines 41-50).

Regarding claim 32, Dong et al teach the use of adenoviral vector, wherein the adenoviral E3 region has been replaced with the *cap* coding region (e.g. Figure 5; Example III).

Regarding claim 33, Dong et al teach the use of adenoviral vector, wherein the *cap* coding region is operably linked to a heterologous promoter such as the E3 promoter or another heterologous promoter such as the CMV early promoter (e.g. column 21, lines 58-67).

Regarding claim 37-39, Dong et al teach a method of producing recombinant AAV (rAAV) virions, comprising the following steps: (i) introducing a recombinant AAV vector into a host cell, (ii) infecting the cell with recombinant adenovirus capable of expressing one or more AAV proteins, and (iii) culturing the cell under conditions and for a period of time effective to allow the cell to produce recombinant AAV virions (e.g. column 7, lines 45-61; Example II). Dong et al teach the introduction of AAV by infection (e.g. column 25, lines 30-61).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Dong et al (US Patent No. 6,686,200; see the entire reference) in view of Johnson (US Patent Application Publication No. 2005/0169892; see the entire reference).

The teachings of Dong et al are described above and applied as before.

Dong et al do not teach the *cap* coding region operably linked to an inducible promoter.

Johnson teaches packaging of AAV using an adenovirus vector where the AAV *rep* and *cap* genes are cloned into the adenovirus genome in the E3 location under the control of the

tetracycline operator (e.g. paragraph [0062]). Johnson teaches that the method provides additional rep and cap genes to the cell line to increase the titer of the RAAV generated (e.g. paragraph [0059]).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Dong et al to include the tetracycline operator taught by Johnson et al because Dong et al teach it is within the ordinary skill in the art to use a variety of heterologous promoter to express the AAV *cap* gene from the deleted E3 region of the adenoviral plasmid vector and Johnson teaches the expression of *cap* from the E3 location using a tetracycline responsive promoter.

One would have been motivated to make such a modification in order to receive the expected benefit of increasing the titer of rAAV produced as taught by Johnson et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Dong et al (US Patent No. 6,686,200; see the entire reference) in view of Massie et al (Biotechnology, Vol. 13, pages 602-608, 1995; see the entire reference).

The teachings of Dong et al are described above and applied as before.

Dong et al do not teach the *cap* coding region operably linked to an adenovirus major late promoter.

Massie et al teach a regulatory sequence comprising a BKV enhancer and the adenovirus major late promoter (e.g. page 607, Expression vector constructions). Massie et al teach the use of the regulatory sequence with an adenovirus plasmid vector that has been introduced into 293 cells (e.g. (paragraph bridging pages 605-606; page 607, Production of Ad recombinant proteins; Figure 1). Further, Massie et al teach that the regulatory sequence is capable of increasing the expression of the operably linked coding sequence such that it is the most abundant polypeptide in the cell (e.g. paragraph bridging pages 602-603).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Dong et al to include the regulatory sequence comprising the adenovirus major late promoter taught by Massie et al because Dong et al teach it is within the ordinary skill in the art to use various heterologous promoters to drive expression of the *cap* gene from an E3 deleted adenoviral plasmid and Massie et al teach it is within the skill of the art to use the adenovirus major late promoter as a heterologous promoter within the context of an adenoviral plasmid vector.

One would have been motivated to make such a modification in order to receive the expected benefit of increased protein expression as taught by Massie et al. One would have been motivated to increase the protein expression of the *cap* gene to increase the production of the rAAV virions. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 35-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dong et al (US Patent No. 6,686,200; see the entire reference) in view of Johnson (US Patent Application Publication No. 2005/0169892; see the entire reference) further in view of No et al (Proc. Natl. Acad. Sci. USA, Vol. 93, pages 3346-3351, 1996; see the entire reference).

The teachings of Dong et al are described above and applied as before.

Dong et al do not teach the *cap* coding region operably linked to an inducible promoter.

Johnson teaches packaging of AAV using an adenovirus vector where the AAV *rep* and *cap* genes are cloned into the adenovirus genome in the E3 location under the control of the tetracycline operator (e.g. paragraph [0062]). Johnson teaches that the method provides additional rep and cap genes to the cell line to increase the titer of the RAAV generated (e.g. paragraph [0059]).

No et al teach an ecdysone-inducible gene expression system for mammalian cells comprising a modified ecdysone receptor and retinoic receptor expressed from a plasmid which has been stably integrated or transiently introduced into a cell line such as a 293 cell in combination with a promoter comprising an ecdysone-response element (e.g. page 3349, Optimization of EcR Derivatives, Construction of a Novel Ecdysone-Specific Response Element; page 3348, Stable Cell Line Production; Figure 2). In comparison to the tetracycline-based inducible promoter system, the system taught by No et al has 500-fold lower basal activity than that of rtTA and induces a 1000-fold increase in gene expression as compared to a 2.5-fold increase induced by rtTA (e.g. page 3350, Comparison of Tetracycline-Based vs. Ecdysone-Inducible Systems). Further, No et al teach that the advantages of an ecdysone-inducible promoter include the use of ecdysteroid, which efficiently penetrates all cells, has a short half-

life, which allows for precise and potent induction, favorable pharmokinetics that prevent storage and expedite clearance, and is non-toxic in cultured mammalian cells (e.g. page 3346, paragraph bridging columns).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Dong et al to include the tetracycline operator taught by Johnson et al because Dong et al teach it is within the ordinary skill in the art to use a variety of heterologous promoter to express the AAV *cap* gene from the deleted E3 region of the adenoviral plasmid vector and Johnson-teaches the expression of *cap* from the E3 location using a tetracycline responsive promoter. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teachings of Dong et al and Johnson to include the ecdysone-inducible promoter in place of the tetracycline-inducible promoter taught by Johnson because Johnson teaches the use of an inducible promoter and No et al teach that the ecdysone inducible promoter can be used as an inducible promoter in cultured mammalian cells.

One would have been motivated to make such a modification in order to receive the expected benefit of increasing the titer of rAAV produced as taught by Johnson. Further, one would have been motivated to replace the tetracycline inducible promoter taught by Johnson with the ecdysone-inducible promoter taught by No et al in order to receive the expected benefit of lower base line expression with a more potent induction that can be better controlled based upon the advantageous pharmokinetic properties of ecdysteroid relative to tetracycline as taught by No et al. Based upon the teachings of the cited references, the high skill of one of ordinary

skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 50 is rejected under 35 U.S.C. 103(a) as being unpatentable over Xiao et al (Journal of Virology, Vol. 72, No. 3, pages 2224-2232, 1998; see the entire reference).

Xiao et al teach a method of producing recombinant AAV (rAAV) comprising introducing AAV vector, AAV packaging plasmid, and Ad helper plasmid into a host cell (e.g. page 2227, right column, last full paragraph; page 2226, Production and measurement of titers of rAAV vectors). Xiao et al teach plasmid pXX2, which is an AAV packaging plasmid that contains the AAV *rep* and *cap* genes (e.g. page 227, right column, last full paragraph; Figure 1A). Xiao et al teach plasmid pXX6, which is an Ad helper plasmid comprising the adenoviral E2a, E4 and VA RNA genes (e.g. page 226, right column, last full paragraph; page 227, right column, last full paragraph; Figure 1B). Xiao et al teach the use of 293 cells, which provide E1a and E1b genes, as packaging host cells (e.g. page 2226, right column last full paragraph). Each of the genes used by Xiao et al have a desirable function for the production of rAAV, such as the stabilization of viral mRNA by E2a (e.g. paragraph bridging pages 2224-2225). Further, Xiao et al teach that the combination of pXX2 and pXX6 contains the essential helper genes but lacks Ad structural and replication genes and results in a 40-fold increase in rAAV compared to conventional procedures that use Ad particles as the helper (e.g. page 2225, paragraph bridging columns).

Xiao et al do not teach the E2a gene in the Ad helper plasmid rather than the AAV packaging plasmid.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to transfer the E2a sequence from plasmid pXX6 to pXX2 of Xiao et al because Xiao et al teach that VA RNA and E2a enhance viral mRNA stability and efficiency of translation (e.g. paragraph bridging pages 2224-2225). After the modification, each plasmid would contain a gene that functions to enhance viral mRNA stability and efficiency of translation. Further, Xiao et al teach it is within the skill of the art to use different combinations and ratios of the factors involved in rAAV virion production (e.g. Figures 1 and 4).

One would have been motivated to make such a modification in order to receive the expected benefit of including E2a at the same copy number as the rep and cap genes in order to provide mRNA stability and efficiency of translation of these viral genes as taught by Xiao et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 50-52 and 54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xiao et al (Journal of Virology, Vol. 72, No. 3, pages 2224-2232, 1998; see the entire reference) in view of Inoue et al (Journal of Virology, Vol. 72, No. 9, pages 7024-7031, 1998; see the entire reference).

The teachings of Xiao et al are described above and applied as before.

Xiao et al do not teach the E2a gene in the Ad helper plasmid rather than the AAV packaging plasmid. Xiao et al do note teach the method further comprising the step of introducing a fourth nucleic acid comprising an SV40 large T-antigen coding region operably

linked to an inducible promoter into the host cell, wherein the third nucleic acid molecule further comprises an SV40 origin of replication.

Inoue et al teach that gene expression from the AAAV packaging constructs is the rate limiting step in vector production, and that published packaging cell lines contain 10 to 30 copies of the *rep* and *cap* genes because they do not amplify further since they lack the AAV TRs required for replication (e.g. page 7024, right column, full paragraph). Inoue et al teach that vector constructs such as plasmids linked to SV40 origins are amplified in the presence of SV40 T antigen (e.g. paragraph bridging pages 7024-7025; page 7026, right column, last full paragraph). Inoue et al teach an AAV packaging plasmid that contains the AAV *rep* and *cap* genes and the SV40 origin (e.g. paragraph bridging pages 7026-7027). Inoue et al teach the construction of packaging cells by transfecting the cells with a plasmid encoding rtTA and selecting cells with high rtTA expression levels (e.g. page 7026, Generation of AAV vector packaging cell lines). Further, Inoue et al teach the transfection of cells with a plasmid encoding the SV40 T-antigen under the control of the *tet* operator (e.g. page 7026, Generation of AAV vector packaging cell lines). Inoue et al teach that the use of the disclosed system results in higher vector titers of AAV vector (e.g. page 7029, paragraph bridging columns). Further, Inoue et al teach that the SV40-based gene amplification method has advantages in that it allows tight regulation of gene amplification to prevent toxic build-up of gene products such as the rep protein (e.g. page 7030, left column, 2nd full paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to transfer the E2a sequence from plasmid pXX6 to pXX2 of Xiao et al because Xiao et al teach that VA RNA and E2a enhance viral mRNA stability and efficiency of translation

(e.g. paragraph bridging pages 2224-2225). After the modification, each plasmid would contain a gene that functions to enhance viral mRNA stability and efficiency of translation. Further, Xiao et al teach it is within the skill of the art to use different combinations and ratios of the factors involved in rAAV virion production (e.g. Figures 1 and 4). Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the 293 packaging cells of Xiao et al to include rtTA expression and a plasmid containing an SV40 T-antigen coding sequence operably linked to the *tet* operator as taught by Inoue et al, as well as including the SV40 origin of replication in the SV40 T antigen plasmid and AAV packaging plasmid comprising the *rep*, *cap* and E2a genes, because Inoue et al teach that the use of the SV40 system to amplify rep and cap gene expression in a controlled manner results in more efficient packaging of rAAV.

One would have been motivated to make such a modification in order to receive the expected benefit of including E2a at the same copy number as the rep and cap genes in order to provide mRNA stability and efficiency of translation of these viral genes as taught by Xiao et al. Further, one would have been motivated to combine the teachings of Xiao et al and Inoue et al in order to receive the expected benefit of increasing the amount of recombinant AAV produced by the system while controlling the expression and toxicity of the *rep* and *cap* genes as taught by Inoue et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 50-52 and 54-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xiao et al (Journal of Virology, Vol. 72, No. 3, pages 2224-2232, 1998; see the entire reference) in view of Inoue et al (Journal of Virology, Vol. 72, No. 9, pages 7024-7031, 1998; see the entire reference) further in view of No et al (Proc. Natl. Acad. Sci. USA, Vol. 93, pages 3346-3351, 1996; see the entire reference).

The teachings of Xiao et al are described above and applied as before.

Xiao et al do not teach the E2a gene in the Ad helper plasmid rather than the AAV packaging plasmid. Xiao et al do note teach the method further comprising the step of introducing a fourth nucleic acid comprising an SV40 large T-antigen coding region operably linked to an inducible promoter into the host cell, wherein the third nucleic acid molecule further comprises an SV40 origin of replication. Xiao et al do not teach the SV40 large T-antigen coding region operably linked to an ecdysone-inducible promoter.

Inoue et al teach that gene expression from the AAAV packaging constructs is the rate limiting step in vector production, and that published packaging cell lines contain 10 to 30 copies of the *rep* and *cap* genes because they do not amplify further since they lack the AAV TRs required for replication (e.g. page 7024, right column, full paragraph). Inoue et al teach that vector constructs such as plasmids linked to SV40 origins are amplified in the presence of SV40 T antigen (e.g. paragraph bridging pages 7024-7025; page 7026, right column, last full paragraph). Inoue et al teach an AAV packaging plasmid that contains the AAV *rep* and *cap* genes and the SV40 origin (e.g. paragraph bridging pages 7026-7027). Inoue et al teach the construction of packaging cells by transfecting the cells with a plasmid encoding rtTA and selecting cells with high rtTA expression levels (e.g. page 7026, Generation of AAV vector

packaging cell lines). Further, Inoue et al teach the transfection of cells with a plasmid encoding the SV40 T-antigen under the control of the *tet* operator (e.g. page 7026, Generation of AAV vector packaging cell lines). Inoue et al teach that the use of the disclosed system results in higher vector titers of AAV vector (e.g. page 7029, paragraph bridging columns). Further, Inoue et al teach that the SV40-based gene amplification method has advantages in that it allows tight regulation of gene amplification to prevent toxic build-up of gene products such as the rep protein (e.g. page 7030, left column, 2nd full paragraph).

No et al teach an ecdysone-inducible gene expression system for mammalian cells comprising a modified ecdysone receptor and retinoic receptor expressed from a plasmid which has been stably integrated or transiently introduced into a cell line such as a 293 cell in combination with a promoter comprising an ecdysone-response element (e.g. page 3349, Optimization of EcR Derivatives, Construction of a Novel Ecdysone-Specific Response Element; page 3348, Stable Cell Line Production; Figure 2). In comparison to the tetracycline-based inducible promoter system, the system taught by No et al has 500-fold lower basal activity than that of rtTA and induces a 1000-fold increase in gene expression as compared to a 2.5-fold increase induced by rtTA (e.g. page 3350, Comparison of Tetracycline-Based vs. Ecdysone-Inducible Systems). Further, No et al teach that the advantages of an ecdysone-inducible promoter include the use of ecdysteroid, which efficiently penetrates all cells, has a short half-life, which allows for precise and potent induction, favorable pharmokinetics that prevent storage and expedite clearance, and is non-toxic in cultured mammalian cells (e.g. page 3346, paragraph bridging columns).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to transfer the E2a sequence from plasmid pXX6 to pXX2 of Xiao et al because Xiao et al teach that VA RNA and E2a enhance viral mRNA stability and efficiency of translation (e.g. paragraph bridging pages 2224-2225). After the modification, each plasmid would contain a gene that functions to enhance viral mRNA stability and efficiency of translation. Further, Xiao et al teach it is within the skill of the art to use different combinations and ratios of the factors involved in rAAV virion production (e.g. Figures 1 and 4). Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the 293 packaging cells of Xiao et al to include rtTA expression and a plasmid containing an SV40 T-antigen coding sequence operably linked to the *tet* operator as taught by Inoue et al, as well as including the SV40 origin of replication in the SV40 T antigen plasmid and AAV packaging plasmid comprising the *rep*, *cap* and E2a genes, because Inoue et al teach that the use of the SV40 system to amplify rep and cap gene expression in a controlled manner results in more efficient packaging of rAAV. Moreover, it would have been obvious to one of ordinary skill at the time the invention was made to regulate the expression of the SV40 T antigen using an ecdysone-inducible promoter because Inoue et al teach the use of an tetracycline inducible promoter and No et al teach that the ecdysone inducible promoter can be used as an inducible promoter in cultured mammalian cells.

One would have been motivated to make such a modification in order to receive the expected benefit of including E2a at the same copy number as the rep and cap genes in order to provide mRNA stability and efficiency of translation of these viral genes as taught by Xiao et al. Further, one would have been motivated to combine the teachings of Xiao et al and Inoue et al in

order to receive the expected benefit of increasing the amount of recombinant AAV produced by the system while controlling the expression and toxicity of the *rep* and *cap* genes as taught by Inoue et al. Moreover, one would have been motivated to use the ecdysone-responsive promoter in place of the tetracycline-responsive promoter in order to receive the expected benefit of lower base line expression with a more potent induction that can be better controlled based upon the advantageous pharmokinetic properties of ecdysteroid relative to tetracycline as taught by No et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 50-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xiao et al (Journal of Virology, Vol. 72, No. 3, pages 2224-2232, 1998; see the entire reference) in view of Inoue et al (Journal of Virology, Vol. 72, No. 9, pages 7024-7031, 1998; see the entire reference) further in view of Nolan et al (US Patent No. 5,830,725; see the entire reference).

The teachings of Xiao et al are described above and applied as before.

Xiao et al do not teach the E2a gene in the Ad helper plasmid rather than the AAV packaging plasmid. Xiao et al do note teach the method further comprising the step of introducing a fourth nucleic acid comprising an SV40 large T-antigen coding region operably linked to an inducible promoter into the host cell, wherein the third nucleic acid molecule further comprises an SV40 origin of replication. Xiao et al do not teach the step of introducing a fifth nucleic acid comprising an Epstein-Barr virus nuclear antigen 1 coding region into the host cell, wherein the third nucleic acid further comprises an Epstein-Barr virus latent origin of replication.

Inoue et al teach that gene expression from the AAAV packaging constructs is the rate limiting step in vector production, and that published packaging cell lines contain 10 to 30 copies of the *rep* and *cap* genes because they do not amplify further since they lack the AAV TRs required for replication (e.g. page 7024, right column, full paragraph). Inoue et al teach that vector constructs such as plasmids linked to SV40 origins are amplified in the presence of SV40 T antigen (e.g. paragraph bridging pages 7024-7025; page 7026, right column, last full paragraph). Inoue et al teach an AAV packaging plasmid that contains the AAV *rep* and *cap* genes and the SV40 origin (e.g. paragraph bridging pages 7026-7027). Inoue et al teach the construction of packaging cells by transfecting the cells with a plasmid encoding rtTA and selecting cells with high rtTA expression levels (e.g. page 7026, Generation of AAV vector packaging cell lines). Further, Inoue et al teach the transfection of cells with a plasmid encoding the SV40 T-antigen under the control of the *tet* operator (e.g. page 7026, Generation of AAV vector packaging cell lines). Inoue et al teach that the use of the disclosed system results in higher vector titers of AAV vector (e.g. page 7029, paragraph bridging columns). Further, Inoue et al teach that the SV40-based gene amplification method has advantages in that it allows tight regulation of gene amplification to prevent toxic build-up of gene products such as the rep protein (e.g. page 7030, left column, 2nd full paragraph).

Nolan et al teach that chimeric vectors containing latent replication functions of the Epstein-Barr virus (Orip/EBNA-1) as well as SV40 origin sequences, replicate predominantly in a lytic mode in the presence of functional SV40 large T-antigen, which allows the amplification of vector copy number above that typically maintained by one origin (e.g. column 7, lines 14-25; Table 3). Nolan et al teach that the EBNA-1 protein provides low copy replication and retention

function (e.g. Table 3). Nolan et al teach that the EBNA-1 protein can be encoded by an episomal vector or can be stably introduced into the host cell prior to transfection (e.g. column 6, lines 42-46).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to transfer the E2a sequence from plasmid pXX6 to pXX2 of Xiao et al because Xiao et al teach that VA RNA and E2a enhance viral mRNA stability and efficiency of translation (e.g. paragraph bridging pages 2224-2225). After the modification, each plasmid would contain a gene that functions to enhance viral mRNA stability and efficiency of translation. Further, Xiao et al teach it is within the skill of the art to use different combinations and ratios of the factors involved in rAAV virion production (e.g. Figures 1 and 4). Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the 293 packaging cells of Xiao et al to include rtTA expression and a plasmid containing an SV40 T-antigen coding sequence operably linked to the *tet* operator as taught by Inoue et al, as well as including the SV40 origin of replication in the SV40 T antigen plasmid and AAV packaging plasmid comprising the *rep*, *cap* and E2a genes, because Inoue et al teach that the use of the SV40 system to amplify rep and cap gene expression in a controlled manner results in more efficient packaging of rAAV. It would have been obvious to one of ordinary skill in the art at the time the invention was made to include the Epstein-Barr virus origin of replication in the third nucleic acid molecule in addition to the SV40 origin of replication because Inoue et al teach it is within the skill of the art to use the SV40 origin of replication for episomal amplification, and Nolan et al teach that the Epstein-Barr virus origin of replication can be used in combination with the SV40 origin of replication for episomal amplification. Further, it would have been

obvious to one of ordinary skill in the art at the time the invention was made to include the plasmid containing an Epstein-Barr virus origin of replication and the EBNA-1 coding region to provide a source of EBNA-1 protein to provide low copy replication and retention of the AAV packaging plasmid and source of EBNA-1 protein.

One would have been motivated to make such a modification in order to receive the expected benefit of including E2a at the same copy number as the rep and cap genes in order to provide mRNA stability and efficiency of translation of these viral genes as taught by Xiao et al. Further, one would have been motivated to combine the teachings of Xiao et al and Inoue et al in order to receive the expected benefit of increasing the amount of recombinant AAV produced by the system while controlling the expression and toxicity of the *rep* and *cap* genes as taught by Inoue et al. Moreover, one would have been motivated to include the Epstein-Barr virus origin in the AAV packaging plasmid and an additional plasmid comprising the EBNA-1 coding sequence and an Epstein-Barr virus origin in order to receive the expected benefit of further amplification of the packaging plasmid as taught by Nolan et al, which would increase the titer of the rAAV virions produces as taught by Inoue et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Conclusion

No claims are allowed.

Claim 40 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The prior art teaches that E1B55k is an essential helper component for AAV replication (Samulski et al, Journal of Virology, Vol. 62, No. 1, pages 206-210, 1988; e.g. page 206, left column, 3rd paragraph). Thus, Applicant's discovery that E1B55k is not required in the context of the disclosed packaging system is unexpected. Therefore, the pending claims that recite the limitation of an accessory vector comprising an adenovirus E1B region lacking an intact E2B55k coding region are free of the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached at 571-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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